

A Modified Catheterization Procedure to Reduce Bladder Damage when Collecting Urine Samples from Holstein Cows

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ABSTRACT. This study proposed a modified procedure, using a small balloon catheter (SB catheter, 45 ml), for reducing bladder damage in cows. Holstein cows and the following catheters were prepared: smaller balloon catheter (XSB catheter; 30 ml), SB catheter and standard balloon catheter (NB catheter; 70 ml, as the commonly used, standard size). In experiment 1, each cow was catheterized. The occurrence of catheter-associated hematuria (greater than 50 RBC/HPF) was lower in the SB catheter group (0.0%, $n=7$) than in the NB catheter group (71.4%, $n=7$; $P<0.05$). In experiment 2, general veterinary parameters, urine pH, body temperature and blood values in cows were not affected before or after insertion of SB catheters ($n=6$). The incidence of urinary tract infection (UTI) was 3.0% per catheterized day ($n=22$). In experiment 3, feeding profiles, daily excretion of urinary nitrogen ($P<0.05$) and rate from nitrogen intake in urine ($P<0.01$), were higher with use of the SB catheter ($n=13$) than with the use of the vulva urine cup ($n=18$), indicating that using the SB catheter can provide accurate nutritional data. From this study, we concluded that when using an SB catheter, the following results occur; reduction in bladder damage without any veterinary risks and accuracy in regard to feeding parameters, suggesting this modified procedure using an SB catheter is a useful means of daily urine collection.

KEY WORDS: feeding profile, Foley catheter, urinary tract infection, urine collection, veterinary profile.

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Collection of the total output of urine is frequently necessary for nutritional studies of cows. However, many urine samples obtained using collection tools, such as vulva urine cups, are often contaminated with hair and feces, which interfere with identification of urinary compounds. Cunningham *et al.* [13] and Crutchfield [12] demonstrated the use of a Foley catheter to collect urine samples from cows. This method yields pure samples consisting almost exclusively of the total output urine. Many nutritional studies of catheterized cows [17, 18, 21] have been reported.

In humans, using a large balloon catheter damages the bladder [27]. The balloon size and its associated risks, which include the incidence of urinary tract infection (UTI), have been well debated [2, 26, 27]. For cows, Crutchfield [12] used a 75 ml balloon, while Cunningham *et al.* [13] used 50–75 ml balloons. Although balloon size has not been widely discussed, a 75 ml balloon is used in many cow nutritional studies [5, 9, 15]. However, catheterization in ruminants often results in the occurrence of UTI [14]. One report describes the administration of penicillin to catheterized cows [36]. Nevertheless, the protective effect against UTI by the administration of antimicrobials was transient

and associated with the emergence of resistant organisms [30]. To the best of the authors' knowledge, no veterinary experimental research has yet been carried out on the catheterization procedure for cows, particularly with regard to the level of bladder damage and incidence of UTI. Furthermore, no available reports describe whether the commonly used 75 ml balloon is appropriate.

For this study, after examination of the veterinary evidence with regard to safety and the risk to catheterized cows, we developed a modified catheterization procedure that is a more powerful tool for nutritional studies. We hypothesized that because the area of contact between the balloon and bladder wall is less for a small balloon than a standard one, using a small balloon might reduce bladder damage. The object of this study for a proposed modified procedure using a small balloon catheter was three-fold: (1) to elucidate the capability of reducing damage to bladders catheterized with a balloon catheter smaller (30 ml and 45 ml) than standard size (75 ml), (2) to investigate the effect of catheterization on veterinary profiles and (3) to investigate the possibility of using the procedure in a nutritional trial.

MATERIALS AND METHODS

This study consisted of three experiments. The purpose of experiment 1 was to clarify whether a smaller balloon catheter can reduce bladder damage. The purpose of experiments 2 and 3 was to determine, if the catheter based on the results of experiment 1 can be used in a nutritional trial requiring daily urine collection. For this purpose, we investigated the

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effect of catheterization on cows' veterinary profiles in experiment 2 and on feeding profiles in experiment 3.

Animals: Holstein cows farmed at the Tokyo Metropolitan Agriculture and Forestry Research Center (Ome, Japan) were used for all experiments. Niigata Agricultural Research Institute Livestock Research Center (Sanjo, Japan) and Aichi Agricultural Research Center (Nagakute, Japan) were used for experiment 3. All cows were individually housed in stalls and had free access to feed and clean, fresh water. Animal care and handling were conducted in accordance with guidelines [24].

Experiment 1: We investigated two parameters: (1) the occurrence of catheter-associated hematuria; and (2) the macroscopic and microscopic observations of the bladder into which 3 sizes of catheters were inserted. For the first parameter, 21 adult non-lactating cows were prepared. We inserted a standard balloon catheter (NB catheter: All Silicone Foley Catheter; 70 ml, 24 French, straight round tip; Create Medic Co., Ltd., Yokohama, Japan), a smaller balloon catheter (XSB catheter: All Silicone Balloon Catheter; 30 ml, 24 French; Nipro, Osaka, Japan) or a small balloon catheter (SB catheter: Norta[®] Foley Catheter; 45 ml, 24 French; BSN Medical, Hamburg, Germany) into the bladders of 7 cows for each type of catheter. The catheters remained in place, and the urine samples were collected for 3 consecutive days. Catheterization was based on multiple techniques [5, 10, 13, 39]. Other specific procedures were as follows: all cows were given a caudal epidural anesthetic of 3–5 ml of 2% lidocaine HCl (Xylocaine[®] Injection; AstraZeneca International K.K., Osaka, Japan) before catheter insertion. After the catheter was inserted, the end of the catheter was connected to the drainage tube. The end of the drainage tube was connected to a urine collection bag (a 45 l vinyl bag) containing 700 ml of 20% (v/v) H₂SO₄. The drainage tube was closely held with a band and adhesion bond at the inguinal region to prevent the weight of the drainage tube from pulling on the catheter. The urine collection bag was changed every day. In this study, if a cow showed discomfort, we removed the catheter and stopped any further the use of that particular type of catheter. Catheter-associated hematuria was checked on the first day (d 1) with the day of catheterization as the urine sampling start day, and four days later (d 4), when the catheter was removed. Urine samples were collected from the end of the drainage tube from the catheter that had been inserted into the bladder on the morning of d 1 and d 4. The red blood cell count per high-power field at a 400 × magnification (RBC/HPF) was measured using urinary sediment [16] from urine samples. For this study, catheter-associated hematuria was defined as greater than 50 RBC/HPF, because 90% of catheterized humans fall under that criterion [25].

For the second parameter, examination of the bladder, one cow bladder was harvested from each group of the first parameter. Thus, all harvested bladders were from cows in the first group. Bladders were harvested after aortic exsanguination under anesthesia induced intravenously with pentobarbital sodium (Somnopentyl[®] Injection; Kyoritsu Seiyaku, Tokyo, Japan). Each bladder was incised at the median line on the anterior side between the apex of the bladder and the external

opening of the urethra. The inside wall of the bladder was macroscopically examined, and sections were taken from the bladder wall for light microscopic examination as well as an examination of a cross section from the bladder wall.

Experiment 2: This experiment was done as two trials under catheter insertion. We examined: (1) the general condition of each cow in the first trial; and (2) incidence of UTI in the second trial. Each trial preceded the nutritional trial, conducted between November 2004 and July 2007, which required the collection of urine. In each trial, catheter insertion and the urine collection period were the same as in experiment 1, and the catheter used was based on the results of experiment 1. The cows were milked twice daily in the milking parlor. Specific catheterization procedures were as follows: before cows were milked, the end of the drainage tube was removed from the urine collection bag, which was clamped shut at approximately 20 cm from the end; then, the end of the drainage tube was sprayed and wrapped in paper towels soaked in 70% ethanol to prevent infection, before the cows were led to the milking parlor [33]. After milking, the interior of the urine collection bag was sprayed with 70% ethanol; then, the drainage tube was reconnected to the bag.

For the first trial, six lactating cows were used. Urine pH, body temperature, blood values and body weight were measured at the pre-catheter period, one week before catheter insertion (–1 w) and during the period that the catheter was in place, between d 1 and d 4. Urinary creatinine was also measured during the catheterized period. Urine samples for pH were collected from spontaneous urination by manual stimulation in the groin once at –1 w and from the drainage tube once in the morning of d 4. Urinary pH was measured immediately after sampling using a pH meter (model F-22; Horiba, Kyoto, Japan). Body temperature was recorded with a rectal mercury thermometer twice a day (8:30 and 13:30) for 3 successive days at –1 w and one day after d 1 (d 2) to d 4 of the catheterized period. The blood samples were collected from the caudal vein once at –1 w and on d 4. The samples were taken using coded heparinized vacuum tubes (Venoject[®] II VP-H100, Terumo Co., Ltd., Tokyo, Japan) containing anticoagulant. The samples were analyzed immediately after sampling for red blood cell count, hematocrit, hemoglobin content and white blood cell count on a well-calibrated cell counter (model PC-608; ERMA, Tokyo, Japan). Body weight was measured once at –1 w and once at d 4. The total urine samples (100 ml) for urinary creatinine were collected every day for three days from the urine collection bag. These total urine samples were frozen at –20°C until analyzed. Urinary creatinine was determined using a commercial kit analyzer (Creatinine-Testwako; Wako Pure Chemical Industries Ltd., Osaka, Japan) based on the Jaffe method [6]. Urinary creatinine per body weight was calculated.

For the second trial, 13 lactating cows were catheterized a total of 22 times (6 cows were catheterized once, 5 cows were catheterized 2 times and 2 cows were catheterized 3 times). When cows were catheterized more than once, a minimum interval of five weeks was used between catheterizations. Incidence of UTI was checked on d 1, d 4, four days (+d 4) and 11 days (+d 11) after d 4 (catheter removal day). Cows

with a positive urinary bacterial culture result before the start of the trial were excluded. Urine samples on d 1 and d 4 were collected once each day from the drainage tube. The samples on +d 4 and +d 11 were also collected using a sterile Nelaton catheter (Urethral catheter; Fujihira Industry, Tokyo, Japan) once each day. If a cow developed a UTI on d 4 or +d 4, the cow was treated with antibiotics every five days, and a urine sample was not collected on +d 11. One month after final antibiotic administration, urine samples were collected from treated cows and cultured to investigate the progress of the UTI. These samples were collected using the same procedure as on +d 4 and +d 11. The dilution culture method was used to count bacteria in urine samples. Aerobic incubation was conducted at 37°C for 18–24 hr. A UTI was defined, if the bacterial colony count for urine samples was greater than 10^2 CFU/ml.

Experiment 3: We explored the effect of different urine collection methods – vulva urine cup (normal method) or catheter – on feeding profiles. This experiment preceded the nutritional trial between February 2005 and May 2006, which required the collection of urine. Thirty-two lactating cows were used. All cows were fed a total mixed ration feed. This feed was adjusted so as to meet the total digestible nutrient requirements for maintenance and lactation in accordance with the Japanese feeding standards for dairy cows [1]. Milking, catheter insertion, catheter type and period of urine collection with catheter or vulva urine cup were the same as in experiment 2. Urine collection took place after diet was fed continuously for three weeks. Eighteen lactating cows were fitted with a vulva urine cup (Sanshin Industry, Yokohama, Japan), and catheters were inserted into 14 lactating cows. The following feeding profiles were measured: body weight, dry matter intake (DMI), milk yield, output of urine and feces, digestibility, urinary total nitrogen and allantoin, nitrogen balance and nitrogen rate. For urine collection with the vulva urine cup, the same volume and concentration of H_2SO_4 was used as for the urine collection bag in experiment 2. The container was removed and reconnected when milking and was changed daily.

Body weight was measured once each on d 1 and d 4. The measuring of DMI and milk yield was the same as in the study by Koga *et al.* [22]. After feces weight was recorded daily, samples were frozen at $-20^\circ C$ until analyzed. Samples of feed, urine and feces were analyzed for total nitrogen in accordance with standard methods [3]. Wet samples were dried for 48 hr at $55^\circ C$, ground and passed through a 1 mm screen. Only fecal samples for total nitrogen were analyzed on a wet basis. Urinary allantoin was analyzed using the Chen method [11].

Data analysis: Data were analyzed using SAS [29]. Statistical significance was defined as $P < 0.05$. In experiment 1, to determine the effect of balloon size, the occurrence of catheter-associated hematuria was analyzed using Fisher's exact two-tailed test. In experiment 2, to compare the differences before and after the catheterized period, data were analyzed using the Wilcoxon test. To determine the differences between catheterized times (1, 2 and 3 times), the incidence of UTI was analyzed using Fisher's exact two-tailed test. In

experiment 3, to evaluate the differences in feeding parameters among sampling methods, data were analyzed using with the SAS GLM procedure.

RESULTS

In every experiment, all cows recovered from anesthesia approximately 3 hr after catheterization. Some cows showed a small amount of urine leakage between the catheter and urinary tract. After 6 hr of catheterization, urine leakage visibly subsided. Thereafter, each experiment was continued for a period of three days. No catheter slipped out of any cow at any time during the catheterized period. However, in experiment 1, the first of the 7 cows who had an XSB catheter inserted manifested discomfort on d 2, so the XSB catheter inserted into this cow was immediately removed. The cows with an inserted SB catheter or NB catheter did not show any signs of discomfort.

Occurrence of catheter-associated hematuria: Urinary red blood cell counts in experiment 1 are presented in Table 1. Five cows showed catheter-associated hematuria of greater than 100 RBC/HPF in the NB group. Four cows showed less than 3 RBC/HPF in the SB group. A cow showed less than 3 RBC/HPF on d 1 and greater than 500 RBC/HPF on d 2 in the XSB group. The occurrence of catheter-associated hematuria at three days after catheter insertion was 5/7 (71.4%) in the NB group and 0/7 (0.0%) in the SB group. The occurrence of catheter-associated hematuria differed significantly between groups ($P = 0.030$).

Macroscopic and microscopic observations: Figure 1 shows the inside wall of the bladder in experiment 1 on d 4. The bladder from the NB group showed bleeding on the interureteric ridge and the urethral crest; hyperemia had occurred at the bladder apex (Fig. 1a). The bladder from the SB group showed bleeding on the interureteric ridge and the urethral crest; the bladder neck had blood spots (Fig. 1b). Figure 2 shows a micrograph of the bladder with the SB catheter. Bleeding was visible in the lamina propria mucosa

Table 1. Results of hematuria testing (RBC/HPF)

	Catheter group	Period	
		d1	d 4
Cow 1	NB	<3	3
Cow 2	NB	<3	45
Cow 3	NB	<3	>100
Cow 4	NB	<3	>100
Cow 5	NB	<3	>100
Cow 6	NB	<3	>100
Cow 7	NB	<3	>500
Cow 8	SB	<3	<3
Cow 9	SB	<3	<3
Cow 10	SB	<3	<3
Cow 11	SB	<3	<3
Cow 12	SB	<3	14
Cow 13	SB	<3	22
Cow 14	SB	<3	36

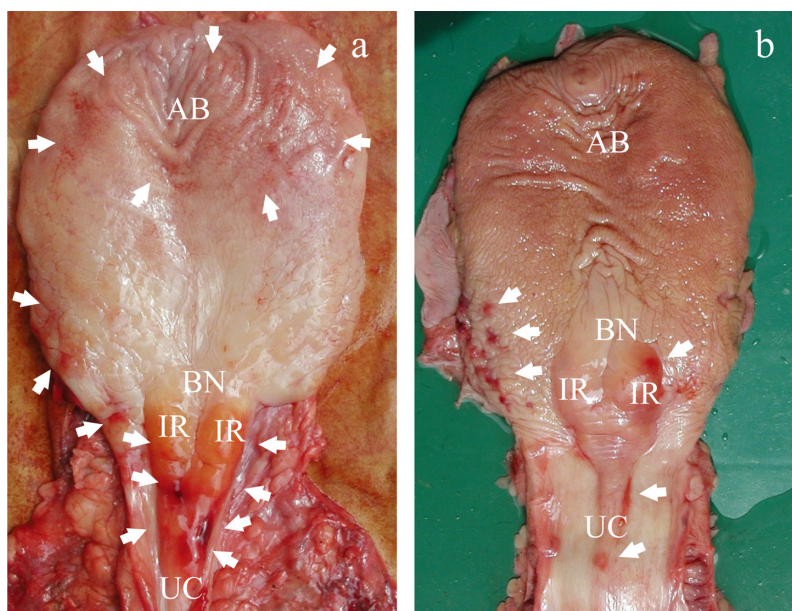


Fig. 1. Macro-scale photograph of a bladder in which the catheter was inserted: (a) bladder in the NB catheter group, (b) bladder in the SB catheter group, (AB) apex of bladder, (IR) interureteric ridge, (BN) bladder neck, (UC) urethral crest. Arrows indicate bleeding.

in the NB and SB group bladders.

General condition: An SB catheter was used in experiment 2 (see discussion). Determined items are presented in Table 2. Urinary pH, body temperature, blood values and body weight did not differ between the pre-catheterized period and the catheterized period ($P > 0.05$). The daily concentration of urinary creatinine was 1.15 ± 0.16 mg/ml and was 21.4 ± 0.7 mg/kg BW per unit of body weight (coefficient value: 3.3%).

The daily excretion of urinary creatinine per unit of body weight was 21.4 ± 0.7 mg/kg BW (coefficient value: 3.3%).

Incidence of UTI: The incidence of UTI did not differ significantly between the catheterized times ($P = 1.000$). The incidence of UTI in experiment 2 is presented in Table 3. Two cows (9.1%) had a UTI on d 4. These two cows with UTI were also UTI positive on +d 4. Other cows without UTI on d 4 did not develop UTI until +d 4 and +d 11. Consequently, the rate of UTI was 3.0% per catheterized day.

The bacterial population density in samples from the two cows with UTI was greater than 10^5 CFU/ml. We administered an antibiotic to these two UTI-positive cows (Mycillin Sol Meiji for Veterinary Use; Meiji Seika Kaisha Ltd., Tokyo, Japan), which yielded effective susceptibility test results. The antibiotic was administered as an intramuscular injection of 20 ml per cow every day for 5 days. Urinary bacteria were not detected in these 2 cows 1 month after injection.

Effect on feeding: An SB catheter was also used in experiment 3 (see discussion). The effect of different urine sampling methods – vulva urine cup or catheter – on feeding profiles is presented in Table 4. Only the output of nitrogen in urine and urinary nitrogen (% of nitrogen intake) differed between sampling methods ($P < 0.05$).

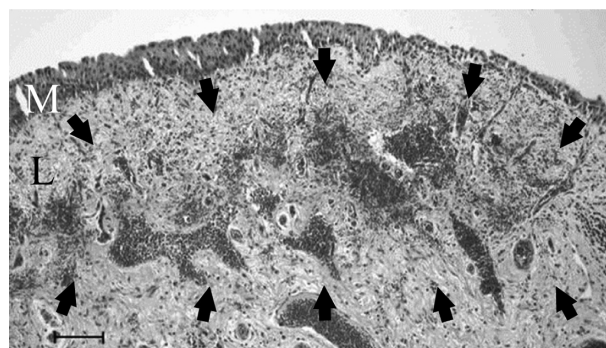


Fig. 2. Micrograph of the cross-section of the SB-catheterized bladder (hematoxylin-eosin stained): (L) lamina propria mucosa, (M) mucosal epithelium. Arrows indicate bleeding. The magnification bar represents $10 \mu\text{m}$.

DISCUSSION

This study proposes a modified procedure using a small balloon for reducing bladder damage when total urine production must be collected from a cow. We hypothesized that a balloon catheter smaller than a standard size catheter (75 ml) can reduce bladder damage. To verify this hypothesis, we inserted three different sizes of balloons (XSB catheter, SB catheter and NB catheter) into cows. Once inserted, the catheters remained in place for 3 days as we compared urinary red blood cell counts and observed the bladder macroscopically and microscopically in experiment 1. When a catheter was determined in experiment 1 to be useful for

Table 2. Effect of catheterization on veterinary general conditions

Items	Period		P-value
	Pre-catheter Median (Mean, Range)	Placing catheter Median (Mean, Range)	
Urine pH	8.70 (8.67, 8.41 to 8.79)	8.61 (8.63, 8.45 to 8.81)	0.438
Body temperature, °C	38.8 (38.8, 38.6 to 38.9)	38.9 (38.9, 38.7 to 39.1)	0.250
Blood items			
RBC, 10 ⁶ /μl	622 (624, 534 to 745)	641 (654, 550 to 799)	0.438
Ht, %	29.2 (29.3, 25.4 to 34.1)	30.2 (29.3, 24.3 to 32.8)	0.844
Hb, g/dl	10.8 (10.9, 9.6 to 11.6)	11.1 (10.6, 8.3 to 12.0)	0.688
WBC, 10 ³ /μl	87 (88, 67 to 106)	91 (90, 69 to 109)	0.688
Body weight, kg	657 (649, 582 to 690)	651 (640, 572 to 687)	0.156
Urinary creatinine, mg/ml	— (—, —)	1.13 (1.15, 0.97 to 1.36)	—
Urinary creatinine, mg/kg BW · day	— (—, —)	21.7 (21.4, 20.4 to 21.9)	—

—: No measure.

Table 3. Incidence of UTI (%)

Item	Period			
	d 1 (n=22)	d 4 (n=22)	+d 4 (n=22)	+d 11 (n=20) ^a
UTI	0.0	9.1	9.1	0.0

a: Number of cows on +d 11 did not include 2 cows that developed UTI on d 4 and +d 4. Those cows were not examined for UTI on +d 11 because they had started antibiotic care after +d 4.

daily collection of urine, we proceeded to experiment 2 for examination of veterinary parameters and to experiment 3 for investigation of feeding profiles.

Comparing size of balloons: In experiment 1, use of a 30 ml balloon catheter was not appropriate as a cow with an inserted XSB catheter showed discomfort. We speculated that the balloon size was too small for a cow's bladder, so the balloon moved location to the vicinity of the urethra for the period of one day. Discomfort to the cow on d 2 probably occurred, because of the balloon irritating the urethra. Balloon catheters of at least 45 ml did not dislocate to urethra, and thus, no irritation occurred there. We therefore decided that the use of an XSB catheter should be discontinued in this study when one of the cows with an inserted catheter showed signs of discomfort. If we had persisted with the use of an XSB catheter during this study, the collection of urine would have failed because of the cows movement due to its discomfort and this study would have been against the animal's welfare.

Macroscopic and microscopic observations revealed that

bladder walls were bleeding or hyperemic in both NB and SB groups. The bladder from the NB group (Fig. 1a) appeared to have heavier bleeding than that from the SB group (Fig. 1b). Under light microscopic observation, bleeding was visible in the lamina propria mucosa in the bladders from the NB and SB groups. The microscopic appearances of the bleeding areas in the bladders were similar for both NB and SB group bladders; Figure 2 depicts a micrograph of the bladder from the SB group. Macroscopic and microscopic observations did not proceed for the XSB group, because only one cow could have the XSB inserted and the catheter would not remain in place for three days. Bleeding or hyperemic areas in bladders from the NB group and the SB group probably occurred when the balloons pressed on the affected areas. The occurrence of catheter-associated hematuria was 71.4% in the NB group and 0.0% in the SB group (significantly different between groups, $P < 0.05$). This demonstrated that the use of SB catheters instead of NB catheters can reduce bladder injury. However, this evidence is inadequate for interpreting the data from only a single bladder per group. The observations in this study indicated that the bladders had been injured by catheterization with NB and SB catheters. It is apparent that catheter-associated hematuria resulted from bladder injury by catheterization. The occurrence of catheter-associated hematuria was significantly lower in the SB group than in the NB group ($P < 0.05$), which supports the hypothesis that using a small balloon might decrease damage to bladders. The difference in volume between the 45 ml and the 70 ml balloons is 25 ml. An NB catheter is 25 g more in weight than an SB catheter. The surface area of an NB catheter is larger than that of an SB catheter. We can be sure

Table 4. Effect of urine sampling method on feeding profiles

Items	Sampling method		P-value
	Vulva urine cup	Catheter	
Body weight, kg	672 ± 16	645 ± 32	0.547
Dry matter intake, kg/d	23.5 ± 0.9	23.4 ± 1.7	0.972
Dry matter intake per body weight, %	3.5 ± 0.1	3.6 ± 0.3	0.767
Milk yield, kg/d	40.6 ± 1.7	34.2 ± 3.4	0.174
Urine output, kg/d	12.0 ± 0.7	15.0 ± 1.4	0.139
Feces output, kg/d	55.0 ± 1.9	51.2 ± 3.8	0.465
Digestible ratio of dry matter, %	66.2 ± 1.0	67.9 ± 2.0	0.533
Digestible ratio of nitrogen, %	63.1 ± 1.3	64.9 ± 2.6	0.627
Urinary nitrogen, mg/ml	9.5 ± 0.6	11.9 ± 1.2	0.145
Urinary allantoin, mg/ml	4.7 ± 0.4	5.7 ± 0.5	0.250
Input and output of nitrogen, g/d			
Intake	554.9 ± 22.9	540.2 ± 46.3	0.817
Milk	197.5 ± 7.1	170.8 ± 14.4	0.187
Feces	203.4 ± 8.1	188.3 ± 16.5	0.505
Urine	114.2 ± 10.1	173.2 ± 20.4	0.044
Retention	39.8 ± 15.4	8.0 ± 31.2	0.458
Input and output of nitrogen, % of nitrogen intake			
Milk	36.3 ± 1.4	31.8 ± 2.9	0.255
Feces	36.9 ± 1.3	35.1 ± 2.6	0.628
Urine	20.5 ± 1.5	33.0 ± 3.1	0.006
Retention	6.3 ± 2.6	0.1 ± 5.2	0.385

that an NB catheter has more potential to cause injury to bladder than an SB catheter. Bladders in the NB group have more potential to be injured than bladders in the SB group, because of the larger area and the stronger pressure of the balloon.

Alternatively, we also have three problems regarding the above consideration. Although Crutchfield [12] proposed using a 75 ml balloon as the standard balloon catheter, we chose to use the NB catheter (a 70 ml balloon) because it was difficult to purchase a 75 ml balloon catheter. Second, we needed to compare the hematuria data between the NB catheter, XSB catheter and SB catheter. The data for the XSB catheter were only taken from one cow, and the data on d 4 could not be obtained because of the cow's discomfort. We decided that comparing only two catheters was the right thing to do. Third, we should compare the hematuria data in this experiment and the normal data. However, since no normal data related to hematuria in cows are expressed in the RBC/HPF ratio, we must discuss the data of the NB group versus the data of the SB group.

In humans, the use of a larger balloon added more weight to the bladder neck [27]. Trauma has been described in catheterized human bladders [27], causing the proliferation of urinary red blood cells [2]. Experiment 1 clarified that the SB catheter was superior to the NB catheter and that the XSB catheter was not appropriate for cows. The NB catheter causes trauma to the bladder and hematuria in cattle, as in humans.

General condition: In experiment 2, all investigated general condition items were not affected between the pre-catheterized period and the catheterized period. The results of urine pH, body temperature and blood values were within

the normal reference range [31]. The daily concentration of urinary creatinine (1.15 ± 0.16 mg/ml) was almost the same as when collected using a vulva urine cup at 8.6 ± 0.6 mg/ml [34]. The daily excretion of urinary creatinine (21.4 ± 0.7 mg/kg BW) was almost consistent with 21.0 ± 1.9 mg/kg BW [34] and 19.6–21.2 mg/kg BW [15]. In spite of this, the data in this experiment are slight lower than 22.6–26.2 mg/kg BW [4]. When compared with other reports, there was no extreme-difference in the concentration or the excretion of creatinine, indicating that renal function did not deteriorate. From these results, we can hypothesize that catheterization does not have a negative effect on the general condition of cows.

Incidence of UTI: In experiment 2, the incidence of UTI with the SB catheter was 9.1% when the catheter remained in place for a period of three days (3.0% per catheterized day). The incidence did not increase during +d 4 and +d 11 when cows that had developed UTI on d 4 and +d 4 were excluded, demonstrating that UTI did not develop after catheters were removed. Moreover, two cows that had shown UTI recovered fully, since no bacteria in urine from those cows were detected after administration of antibiotics.

We can therefore conclude the following: The incidence of UTI with the SB catheter was 3.0% per catheterized day. Cows that develop UTIs can be cured with antibiotics.

Four issues arise regarding the incidence of UTI. First, 7 of 13 cows were catheterized more than once, when in reality they should have been catheterized only once. Seven cows had a minimum non-catheterized period of five weeks. The incidence of UTI did not differ significantly between catheterized times ($P > 0.1$). We believed this non-catheterization period cleared up the affects of the catheter insertions upon the cows that had been catheterized more than once.

Therefore, the incidence of UTI was computed as if 22 cows had been catheterized. Second, UTI was defined strictly in terms of the urinary bacterial count in this experiment. Van Metre [14] described hematuria consistently resulting from UTI in cows. Evidence of a relationship between bacteriuria and hematuria was not obtained in this experiment, because hematuria in cows with UTI (bacteriuria positive) was not monitored. We assume that a few cows without UTI would have a positive hematuria test after catheterization, because of the maximum of 36 RBCs/HPF found in urine samples collected using the SB catheter on d 4 in experiment 1 (Table 1). Therefore, detection of bacteriuria is more adequate than detection of hematuria. Third, the incidence of UTI lasted only for a period of three days in this experiment. The incidence of UTI under catheterization for periods of more or less than three days is undefined. Three days' catheterization is an appropriate period, because almost all cow catheterization periods reported in the literature were 3 days [9, 21, 36, 38]; some other cases comprised 4 days [19] and 6 days [18]. Moreover, significant risk factors for developing UTI were increased during catheterization in humans [28] and in dogs [30]. The infecting organisms form a biofilm on the catheter surface, and these encrustations provide a refuge for bacteria that are very resistant to antibiotics [23]. Fourth, the incidence of UTI with the NB catheter was not determined. From the result of experiment 1, the NB catheter causes more trauma than the superior SB catheter. Given the risk of UTI with the NB catheter, its use should not be encouraged. It should be noted that this study is the first to show the incidence of UTI in cows. For that reason, we sought to compare our data against other data, such as those for humans and dogs. The incidence in this experiment (9.1%) was subequal with studies in humans, 8.7% [7] and 9.1% [20] for catheterization over a period of three days. Furthermore, the incidence of UTI was 3.0% per catheterized day in this study, which was consistent with the rate in humans [39], 3–10%, and in dogs [30], 4.7% per catheterized day. Because the incidence of UTI in SB-catheterized cows was not higher than other data, it is apparent that handlers should not be concerned about the urinary tract infection risk in SB-catheterized cows.

Before concluding this section, we note that many studies on the prevention of UTI have presented data for humans [8, 32, 35, 37]. However, UTI prevention techniques have not been successfully developed. We strongly recommend maintaining the hygienic conditions set forth in the CDC guidelines [10] and surveying animals to detect UTI before and after catheterization. Furthermore, the length of time for catheterizing cows should be as brief as possible in trials to avoid increasing bacterial mobility.

Effect on feeding: In experiment 3, the results of feeding profiles show that feeding data, without the nitrogen output in urine and the urinary nitrogen (% of nitrogen intake), were not influenced by the two types of collection methods – vulva urine cup and catheter. We can offer little explanation why the nitrogen output in urine and the urinary nitrogen (% of nitrogen intake) differed between sampling methods. Urinary creatinine concentration was almost the same as it is when collected using a vulva urine cup (see general condi-

tions in the discussion). This means that inserting catheters into the bladder does not increase urine output. There was no difference in urine output or urinary nitrogen concentration when using either of these sampling methods. However, the nitrogen output in urine and the urinary nitrogen (% of nitrogen intake) did yield higher values when using the catheter as opposed to the vulva urine cup. These differences according to sampling method can be explained by assuming that the degree of urine collection was higher for catheters and thus the loss of urinary nitrogen was lower for catheters. We suggest that there is a significant difference when using an SB catheter in the detection of urinary data in a nutritional trial. In addition, since the daily output of urine did not differ between sampling methods, total urine collection during a 3-day catheterized period was successful without urine loss and was useful for total urine collection.

Conclusion: Results show that a 30 ml/ balloon catheter is not appropriate for urine collection in cows. This study proposes a modified catheterization procedure using a 45 ml/ balloon. The procedure is more useful than a previous procedure using a 75 ml/ balloon catheter. The procedure has three key elements: reduced bladder damage, 9.1% infection (UTI) risk per three continuously catheterized days and problem-free total urine collection for nutritional trials. In the future, accurate total urinary data related to nutrition and feeding can be obtained and considered using this modified procedure which presents a starting point for a new line of research.

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